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Jain-Schlaepfer, Sofia; Midwood, Jonathan D.; Larsen, Martin Hage; Aarestrup, Kim; King, Greg; Suski, Cory; Cooke, Steven J.

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S.M.R. Jain-Schlaepfer*[a], J.D. Midwood[b], M.H. Larsen[cd], K. Aarestrup[c], G.D. King[e], C.D. Suski[e] and S.J. Cooke[a]

[a]Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, 1125 Colonel By Dr. Ottawa, ON K1S 5B6, Canada

[b] Great Lakes Laboratory for Fisheries and Aquatic Science. Fisheries and Oceans Canada, Burlington, ON, L7S1A1, Canada

[c] National Institute of Aquatic Resources, Freshwater Fisheries, Technical University of Denmark, Vejlsøvej 39, 8600 Silkeborg, Denmark

[d] Danish Centre for Wild Salmon, Brusgårdsvej 15, DK-8960 Randers SØ, Denmark.

[e]Department of Natural Resources and Environmental Sciences, University of Illinois, 1102 S. Goodwin Ave, Urbana, IL 61801, USA.

*sofiaj@rogers.com, 0467694922, 32 McKinley Street, North Ward 4810, Australia

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22 Cooke

23 ***Abstract***

24 There is considerable variation in individuals' glucocorticoid (GC) baseline status and stress
25 responses, yet the cause and consequence of this variation remains ambiguous. Attempts to
26 relate GC levels to fitness and life-history tradeoffs have yielded variable results. In this study,
27 we evaluated whether baseline and post-stressor GC hormone concentrations predicted
28 migration strategy (i.e., resident or migrant) and successful seaward migration in a partially
29 migrating population of juvenile brown trout (*Salmo trutta* (Linnaeus, 1758)). Baseline (*N*=99)
30 or post-stressor (*N*=102) plasma cortisol concentrations were obtained from trout and they
31 were tagged with passive integrated transponder (PIT) and released in a natural Danish stream.
32 Subsequently, fish were tracked with PIT reader systems and the stream was resampled for
33 resident individuals. GC levels were not found to be associated with recapture of resident
34 individuals or migration propensity to our first tracking station (S1), but increased baseline (and
35 not post-stressor) GC levels were associated with increased passage from S1 to our second
36 tracking station, which anecdotally was an area of high predation or challenge. Our study found
37 no evidence to suggest that cortisol regulates the migration life-history in juvenile brown trout,
38 but intermediate increases in baseline GC (and not post-stressor GC) levels may favor migration
39 performance.

40

Key Words

Cortisol, Fish, Glucocorticoid, Migration, Stress response, Brown trout, *Salmo trutta*

Abbreviations

GC, glucocorticoid; PIT, passive integrated transponder; S1, tracking station 1; S2, tracking station 2; HPA, hypothalamic–pituitary–adrenal axis; HPI, hypothalamic–pituitary–interrenal axis; K, Fulton's condition factor.

48 ***Introduction***

49 The study of the physiological basis of stress is useful for understanding physical, chemical,
50 social and perceived challenges faced by organisms, and the functional responses employed by
51 organisms to deal with those stressors (Denver 2009). In vertebrates, real or perceived
52 stressors stimulate the hypothalamic–pituitary–adrenal axis (HPA; or the hypothalamic–
53 pituitary–interrenal (HPI) axis in fish) resulting in the release of glucocorticoid (GC) hormones
54 into the blood stream (Sapolsky et al. 2000; Barton 2002). Thus, circulating concentrations of
55 GC hormones are often measured to understand an organism's stress response (Dantzer et al.
56 2014). Within a species, individuals often show repeatable baseline (prior to a stressor) GC
57 levels and a repeatable GC response following a stressor (Pottinger and Carrick 2001;
58 Ouedraogo et al. 2004; Wada et al. 2008; Rensel and Schoech 2011; Cook et al. 2012; Narayan
59 et al. 2013; Love et al. 2015) resulting in different reactive scope profiles (difference between
60 GC response and baseline) between individuals (Romero et al. 2009). The biological
61 functionality, as well as the individual and evolutionary consequences of such variability among
62 individua, are still debated (Breuner et al. 2008; Bonier et al. 2009).
63
64 To define the biological consequences of individual variation in GC levels during the stress
65 response, researchers have attempted to relate inter-individual differences in baseline and
66 post-stressor GC concentrations to performance (e.g., migration, parental care, territorial
67 behavior) and fitness (e.g. Comendant et al. 2003; Cabezas et al. 2007; Breuner et al. 2008).
68 From a theoretical point of view, it is often hypothesized that individuals with greater post-
69 stressor GC levels should have increased long term fitness (Breuner et al. 2008), because an

individual's stress response increases chances of surviving the stressor (e.g. predation attempt) and mediates trade-offs between reproduction and survival. Indeed, experimental studies show variability in the relationship between the magnitude of the stress response and performance or fitness measures (reviewed by Breuner et al. 2008). This relationship is increasingly understood as context dependent, because an elevated stress response in an emergency situation shifts resources from reproduction to survival (Wingfield et al. 1998); however, an elevated stress response when the perception of stressors is exaggerated can lead to the misallocation of resources, thereby limiting investment in growth and reproduction (Hawlena and Schmitz 2010). There is a paucity of studies directly examining inter-individual differences in stress response while concurrently measuring performance or fitness (Romero and Wikelski 2001; Blas et al. 2007; Cook et al. 2011).

In contrast to the theorized positive association between elevated post-stressor GC levels and performance or fitness, higher baseline GC levels are often inferred to indicate reduced condition, because it suggests an organism is experiencing greater environmental stressors (Cort-Fitness hypothesis; reviewed by Bonier et al. 2009). Higher GC levels are in circulation when exposed to a stressor and are associated with the reallocation of resources from fitness-related factors, such as growth and reproduction, towards the restoration of physiological homeostasis. Despite the potential negative consequences associated with higher baseline GC levels, some studies have found that moderate long term increases in GCs can actually increase individual performance (Comendant et al. 2003; Pravosudov 2003; Cabezas et al. 2007). Further

investigation is therefore warranted to understand the relationship between baseline GC hormones and performance.

Both baseline and post-stressor GC concentrations have a heritable component in some species (Evans et al. 2006; Jenkins et al. 2014) and are associated with a suite of other traits that can lead to different life history and coping strategies (Koolhaas et al. 1999; Pottinger and Carrick 1999; Blas et al. 2007; Øverli et al. 2007; Wada et al. 2008). Selection on heritable variation in GC levels and response is thought to drive the evolution of life-history traits (Bolnick et al. 2003; Williams 2008), such as migration behavior. Migration in particular, requires large amounts of energy and often exposes individuals to many stressors including predation and changes in environmental condition (Dingle 2014). Thus, GC levels have the potential to be an important factor determining successful migration (Holberton 1999; Piersma et al. 2000; Angelier et al. 2009), which could affect fitness and potentially drive the evolution of migration strategies. Migration success, meaning successfully reaching the migration destination, has been linked to GC levels in a single species of fish, adult sockeye salmon (*Oncorhynchus nerka* (Walbaum, 1792)), where upriver migration success was found to increase with stress responsiveness but not baseline stress levels (Cook et al. 2014). GC levels have also been related to the smoltification process in anadromous salmonids. Smolting fish generally have higher baseline and peak GC, which appears to induce the physiological changes required to cope with the transition from freshwater to saltwater (e.g., increased osmoregularity capacity; Barton et al. 1985; Redding et al. 1991; Björnsson et al. 2011). Stressors that cause a GC response may cause a reallocation of energy towards immediate survival resulting in a change in life history and

coping strategy (Landys et al. 2006; Shepard et al. 2008). Thus, GC variation resulting from past or current conditionsmay influence the "decision" to migrate. Alternative migration strategies have the potential to be heritable through heritable GC regulation (Zera and Harshman 2001; Williams 2008).

For our study species, brown trout (*Salmo trutta* (Linnaeus, 1758)), migration success provides a measure of performance, and also acts as an indicator of alternate life history strategies. More specifically, European populations of brown trout show a continuum of migration strategies, where subpopulations are capable of anadromous activity, smaller scale migrations within freshwater systems (potamodromous), and residency in natal streams (Cucherousset et al. 2005). This phenomenon is termed partial migration and reflects the fact that only a component of the population engages in migration, a common trait among some salmonid species (Jonsson and Jonsson 1993). Previous studies on the propensity of migration in brown trout have found that individuals with high metabolisms and high growth rates tend to migrate, likely due to higher resource requirements (onsson and Jonsson 1993; Forseth et al. 1999).

The goal of this study is to define relationships between GC levels (baseline and post-stressor), and migration propensity in juvenile brown trout. Given the role of GCs in smoltification, we would expect to see higher post-stress and baseline GC levels in migrating individuals. The effects of individual body condition and size (length, mass) in relation to migration propensity will also be quantified.

Methods

Experimental Design

Brown trout were captured using backpack electroshocking (Scubla ELT 60 II GI; run at 300 volts) in four regions of the Gudsø Stream, Jutland, Denmark (Figure 1) between 11 March and 18 March 2013. This time period was selected as it fell prior to the spring migration, which typically occurs in mid-April (Midwood et al. 2014). Individuals between 120 and 182 mm in total length (TL) were used to minimize the effects of allometry on results. To evaluate an individual's resting plasma cortisol levels (baseline), blood was collected (~0.2-0.3 ml) from a subset of fish within 3 min of capture via caudal puncture of the haemal arch using a 1 ml syringe and 25-gauge needle. A separate subset of fish (Table 1) were exposed to a standardized 3 minute air exposure and then held in a 20 L container with 5 L of freshwater for 30 min prior to the collection of a post-stressor blood sample. Blood was sampled in the same manner as above at 30 ± 3 minutes post-air exposure corresponding with the approximate peak in GCs in brown trout (Pickering and Pottinger 1989). Blood samples were held in a water-ice slurry for no more than 2 hrs until centrifuged for 5 min (10,000 rpm) to isolate plasma. Plasma isolate was decanted, flash frozen and stored (-80˚C) for later analysis of plasma cortisol concentrations. Total length and wet mass of each individual were then measured. Next, a uniquely coded 23 mm PIT tag (RI-TRP-RRHP, half duplex, 134 kHz, diameter 3.85 mm and a mass of 0.6 g in air; Texas Instruments, Plano, Texas, USA) was inserted into the body cavity through a small incision made with a scalpel. Previous tag retention studies on salmonids have found negligible effects on survival and growth (Larsen et al. 2013) and generally low PIT tag rejection rates (< 4% after seven months; Ombredane et al. 1998). Following tagging fish were

157 then placed in a 60 L container of fresh river water and monitored for a minimum of 30 minutes
158 to ensure recovery before they were ultimately released back into the stream near their point
159 of capture. All fish recovered. Length and mass were used to calculate Fulton's condition factor
160 (*K*) for each sampled fish where K = mass (g) × 100 / length^3 (cm) (Ricker 1975).
161
162 *Cortisol Analysis*
163 Plasma cortisol (ng mL^{-1}) was quantified using a commercially available enzyme linked
164 immunosorbant assay (ELISA) (Enzo Life Sciences, Cortisol EIA Kit [901-071], BioAssay Systems)
165 previously validated for use in fishes (Sink et al. 2008). This assay has a detection limit of 0.0567
166 ng mL^{-1}, therefore, the 5 samples with undetectable cortisol concentrations were assigned
167 values equal to this detection limit (Haddy and Pankhurst 1999; Liss et al. 2013).
168
169 *Tracking*
170 To track movements of individuals from their point of release to the sea, two tracking stations
171 were placed approximately 1 km upstream of Kolding Fjord on Gudsø Stream, which was the
172 closest feasible place to the ocean. The first station (S1) was placed upstream of a millpond and
173 the second station (S2) was placed downstream of the millpond, approximately 150 m from S1.
174 Each station was comprised of a paired set of PIT gates spaced approximately 10-m apart. This
175 allowed for an assessment of the direction of movement for individuals at each station. The
176 same array was used by Midwood et al. (2015) and found to have a detection efficiency at S1 of
177 96.3%; detection efficiency could not be evaluated at S2, but missed-detections were assumed

178 to affect all trout equally and therefore should not bias conclusions related to baseline GC or
179 post-stressor GC correlations with relative migration success to S1 and S2.
180
181 To estimate the proportion of individuals that stayed resident and did not migrate to the ocean,
182 the stream was re-sampled using back-pack electrofishing on 18 and 19 June, 2013, at which
183 point migratory individuals should have left the stream. Surveys started near the antenna and
184 proceeded upstream through all areas where brown trout were initially captured (Figure 2). It
185 was not possible to resample the entire system as it is greater than 16 km in length; however,
186 given the shallow and narrow nature of the stream and the relatively high efficiency of capture
187 for salmonid species using electrofishing (Kennedy and Strange 1981; Haynes and Baird 1994),
188 efforts were likely sufficient to provide a relative estimate of residency.
189
190 *Statistical Analyses*
191 Multiple linear regression was used to quantify relationships between plasma cortisol
192 concentrations (for both post-stressor and baseline groups) and timing of migration (time from
193 sampling to S1 passage), while controlling for *K*, length or mass. Assumption of homogeneity of
194 variance and normal distribution for the models were ensured by visual inspection of residual
195 plots. If violated, the response variable was log or square root transformed. Multiple binomial
196 logistic regression was used to test for the effect of plasma cortisol concentration (baseline and
197 post-stressor) on the probability of successful migration to S1 (used as a proxy of migration
198 strategy) and on the probability of successful migration to S2 given successful passage of S1
199 (used as a measure of migration success through the millpond for migrating individuals). Again,

200 separate tests were conducted using mass, length or *K* as a covariate in the model. Length,
201 mass and *K* were also tested separately for effects on all migration response variables with the
202 baseline and post-stressor fish subsets pooled. Two outliers were removed before analyses as
203 they were outside the standard deviation of the mean: a baseline cortisol value of 44.5ng/mL
204 and a peak cortisol value of 3.43ng/mL. All significant results found without the outliers were
205 also found when the outliers were included in the analyses.
206
207 Variation in association with recorded mean values is given as standard error (± SE) throughout.
208 Statistical significance for all analyses was set at $\alpha = 0.05$ and all statistical analyses were
209 conducted in R (v 3.2.2; R Core Team, 2016).
210
211 ***Results***
212 Of the 198 fish tagged, 19 were recaptured in mid-June, 83 were detected passing S1 and 45
213 passing S2 (Table 2). All fish detected at S2 were also detected at S1, suggesting a detection
214 efficiency of 100% at S1. Mean baseline cortisol was 5.6 ± 6.7 ng/mL and post-stressor cortisol
215 was 69.2 ± 40.1 ng/mL (Table 1). Post-stressor (Multiple logistic regression: $Z_{2,96} = 1.44$, $p =$
216 0.15) and baseline (Multiple logistic regression: $Z_{2,96} = 0.15$, $p = 0.89$) plasma cortisol
217 concentrations did not have a significant effect on the probability of successful passage at S1
218 when controlling for K, mass, or length ($p>0.1$; Figure 2b and 2e). For fish that passed S1, the
219 probability of successful passage to S2 increased with increasing individual baseline cortisol
220 when controlling for mass (Multiple logistic regression: $Z_{2,27} = 1.96$, $p = 0.05$) and length
221 (Multiple logistic regression: $Z_{2,27} = 2.01$, p = 0.045), but not for *K* ($p>0.05$). For fish that passed

S1, the probability of successful passage to S2 showed no relationship with post stressor cortisol (All: $p>0.1$)

Mean migration time from blood sampling to S1 passage was 33.4±19.1 days and mean time from S1 to S2 was 3.2±7.6 days. Multiple regression showed no significant relationship between migration duration or date of passage, and post-stressor or baseline cortisol when controlling for mass, length or *K* (All: $p>0.1$). Migration time decreased with increasing fish length (Linear regression: $F_{1,79}= 6.82$, p = 0.01, $R^2 = 0.8$) and fish mass (Linear regression: $F_{1,79} = 4.4$, $p = 0.04$, $R^2 = 0.05$), but no relationship was found with *K* (Linear regression: $F_{1,79}= 3.14$, $p = 0.08$, $R^2 = 0.04$).

Multiple logistic regression revealed no effect of post-stressor and baseline cortisol on the probability of recapture in resident streams when controlling for K, length or mass (Figure 2; All: $p>0.1$). Length, mass and *K* also were found to have no effect on the probability of recapture in resident streams (All: Logistic regression: $p>0.1$). No relationship was found between *K*, mass or length and baseline or post-stressor cortisol (All: Linear regression: $p>0.1$). The probability of S1 passage and the probability of passage between S1 and S2 had no relationship with *K*, fish length and fish mass (All: $p>0.08$).

Discussion

As previous studies have found cortisol to be elevated during smoltification (Barton et al. 1985), we expected individual brown trout with higher baseline and post-stressor cortisol to have a

244 higher probability of migrating downstream to S1 and S2, coupled with a lower probability of
245 recapture as resident. However, no such relationships were found.
246
247 We found no evidence suggesting that migration is correlated with endocrine regulation in
248 juvenile brown trout, despite several studies having documented that cortisol levels rise during
249 smoltification in andromous fish (Barton et al. 1985; Redding et al. 1991; Björnsson et al. 2011),
250 and also that GC regulation has the potential to play a role in determining alternative coping
251 strategies (Zera and Harshman 2001; Landys et al. 2006; Williams 2008). Mass, length and body
252 condition were also not found to have a relationship with plasma cortisol (baseline or post-
253 stressor). Fish of greater mass and length (but not condition) were found to have shorter
254 migration times similar to previous findings (Bohlin et al. 1996). Body condition, length and
255 mass were not found to affect migration propensity to S1. the lack of correlation between our
256 condition metrics and migration propensity is counter to previous findings of reduced condition
257 leading to increased migration propensity (Peiman et al. 2017); however, it is possible that the
258 comparatively limited recapture rate in the present study (*N*=19) prevented the detection of a
259 size-based effect.
260
261 Baseline, but not post-stressor, cortisol concentration was found to be positively associated
262 with passage through the millpond (i.e., from S1 to S2). Previous studies have found brown
263 trout baseline cortisol without chronic stressors to be 0-5 ng/mL, while those exposed to
264 chronic stressors such as confinement or crowding have shown baseline cortisol elevated to 10
265 ng/mL (Pickering and Pottinger 1989). The average baseline cortisol found in individuals

266 recaptured in resident streams, individuals that passed S1 and individuals that passed S2 was
267 5.6 ng/mL, 5.8 ng/mL and 8.4 ng/mL, respectively (Table 2). Thus, individuals that passed
268 through the millpond had baseline cortisol levels of individuals approaching chronically stressed
269 levels on average, while other fish had cortisol levels closer to what would be expected for fish
270 not showing chronic stress.
271
272 The distance between S1 and S2 is only 150 m, thus it is unlikely that S1 versus S2 passage
273 would be indicative of migration strategy. The millpond may therefore be an area of increased
274 mortality, suggesting S1 passage is more indicative of migration strategy (resident and
275 potamodromous versus anadromous) while passage between S1 and S2 is more indicative of
276 migration success of migrant individuals. Forty five percent of all brown trout that passed S1
277 were not detected passing S2, which is only 150 meters downstream, and those that did
278 migrate through the millpond, took much longer than when migrating through the stream,
279 taking an average of 3.2 days to move 150 meters. Similar patterns are found in other studies of
280 migrating fish at Gudso (Midwood et al. 2014, 2015). This indicates the millpond was
281 challenging for migrating trout. We suggest that many of these individuals likely suffered
282 mortality, as it is unlikely for such a large proportion of migrants to take up residency in such a
283 small area. Furthermore, there is anecdotal evidence of high concentration of foraging grey
284 heron (*Ardea cinerea* Linnaeus, 1758) and other birds at the outflow of millpond. Previous
285 studies on salmonid smolts have documented large losses resulting from predation in lakes
286 (Jepsen et al. 1998; Koed et al. 2006) and at weirs and related millponds (Aarestrup and Koed
287 2003). For example, Jepsen et al. (1998) found 90% of smolts died while passing through Lake

Tange, Denmark, with 56 % of the mortality cause by pike (*Esox lucius* (Linnaeus, 1758)) and 31% by birds. Another possibility for the reduced passage and long migration time through the millpond is that there is only one small exit and trout may have had a hard time getting out. While it is possible that overall lower passage at S2 is driven by reduced detection efficiency at this station, these missed-detections would affect all trout equally and thus would not affect the relationship found between baseline cortisol and passage between S1 and S2 other than by reducing statistical power. Our results, therefore, do not support the link between GC levels and migration strategy in brown trout, but they do suggest that baseline GC (and not post-stressor GC) is positively associated with migration success.

Generally it is hypothesized that increased baseline GC are associated with decreased survivorship because increased baseline GC are associated with increased chronic stressors (Cort-Fitness hypothesis: Bonier et al. 2009). Our findings appear to support the opposite trend as migratory fish with lower baseline cortisol appear to have lower successful passage through the millpond (Figure 2). Several other studies have also found higher baseline cortisol to be correlated with increased survivorship: Cabezas et al. (2007) found moderately elevated corticosterones were associated with decreased body condition but increased probability of survival in European wild rabbits (*Oryctolagus cuniculus* (Linnaeus, 1758)); Comendant et al. (2003) found higher corticosterone in female lizards (*Uta stansburiana* (Baird and Girard, 1852)) to be associated with increased survival to second ovulation; and Cote et al. (2006) found increased corticosterone to modify behaviour (locomotor and social activity), increasing survival in male adult common lizards (*Lacerta vivipara* (Jacquin, 1787)).

310 Another possible explanation for why our results are not consistent with the Cort-Fitness
311 hypothesis is that it is difficult to discern whether higher baseline cortisol indicates exposure of
312 the individual to greater chronic stress (which is assumed to be the case by the Cort-Fitness
313 hypothesis; Bonier et al. 2009), or indicates a more competent ability to cope with chronic
314 stressors. The first would likely lead to decreased survivorship with increasing baseline GC level,
315 while the later would likely result in the opposite trend. In our study, all individuals may be
316 subjected to similar chronic stressors as they were all reared in the same stream reach. Thus,
317 baseline stress may be a better indicator of better ability to cope with chronic stressors than
318 exposure to greater environmental stressors.
319
320 Post-stressor cortisol, a proxy for stress responsiveness, was not found to be a significant
321 predictor of migration timing, survival or success. Potentially, the effect of stress
322 responsiveness was masked, because we were not able to control for individual variability in
323 baseline as fish were too small to obtain both a baseline and a post-stressor cortisol sample.
324 However post-stressor cortisol was more variable (SD=40.7 ng/mL) than baseline cortisol
325 (SD=7.7 ng/mL). Cockrem (2013) reviewed studies on baseline and stress-induced GCs and
326 determined that there tended to be more variation in baseline GCs among individuals than in
327 stress-induced levels. Although our findings are not consistent with that generalization,
328 Cockrem (2013) did note that it was not ubiquitous with a number of exceptions. It is unclear
329 why that pattern was observed here but reasons, among others, could include inter-individual
330 differences in how the stressor was perceived or different stress histories. Despite the widely
331 accepted belief that acute stress responses are adaptive, similar to our study several other

studies have failed to find a relationship between stress responsiveness and performance (Romero and Wikelski 2001; Blas et al. 2007), or may even reduce performance. These results may be an indication that different reactive scopes profiles may allow individuals to adequately cope with stressors. Additionally, the benefits of stress responsiveness are increasingly viewed as context dependent, as traits induced by a stress response can have variable consequences to an organisms' performance depending on the nature of the stressor (Breuner et al. 2008).

Similar to our findings in brown trout, the only other study we are aware of that examines individual variation in endocrine regulation in relation to migration in fish (anadromous semelparous sockeye salmon) found no correlation between GC levels and migration timing or rate. They also found individuals with higher stress responsiveness to have greater migration success, but that baseline cortisol not to be a significant predictor of migration success (Cook et al. 2014). Differences in the relationship between GC secretion and migration success found by our study may be attributed to differences in the stressors experienced. For example, salmon may have more chronic stressors such that their baseline levels are at the turning point of the baseline cortisol-survivorship curve hypothesized by Cabezas et al. (2007), where differences in baseline levels have negligible effects on survivorship. The inconsistency between findings may also be reflective of differences in physiology between species.

Conclusions

353 In summary, we found no evidence suggesting that cortisol regulates the migration life history
354 trade off in juvenile brown trout. But, slightly higher levels of baseline GC (and not post-stressor
355 GC) may favor migration performance. Further study is warranted to help determine the
356 relationship between migration success and GC, with special focus on stress responsiveness
357 within an individual.
358
359
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368
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549 Table 1: The mean (with SD) and range of plasma cortisol, individual fish length, mass and
550 Fulton's Condition factor (*K*), for juvenile brown trout (*Salmo trutta*) collected from the Gudsø
551 Stream, Denmark. Collected fish were blood sampled either within three minutes of capture
552 (baseline) or 30 minutes following a standardized three minute air exposure (post-stressor).

Treatment	Baseline	Post-Stressor
Sample size	99	99
Cortisol (ng/mL)	5.6 ± 6.7	69.2 ± 40.1
	(0.1 to 25.5)	(7.2 to 197.6)
Length (mm)	146±126	147±13
	(120 to 182)	(122 to 171)
Mass (g)	28.2±7.6	29.2±8.1
	(15.8 to 48.5)	(17.1 to 45.8)
K	0.90±0.07	0.90±0.06
	(0.66 to 1.06)	(0.72 to 1.09)

553

554

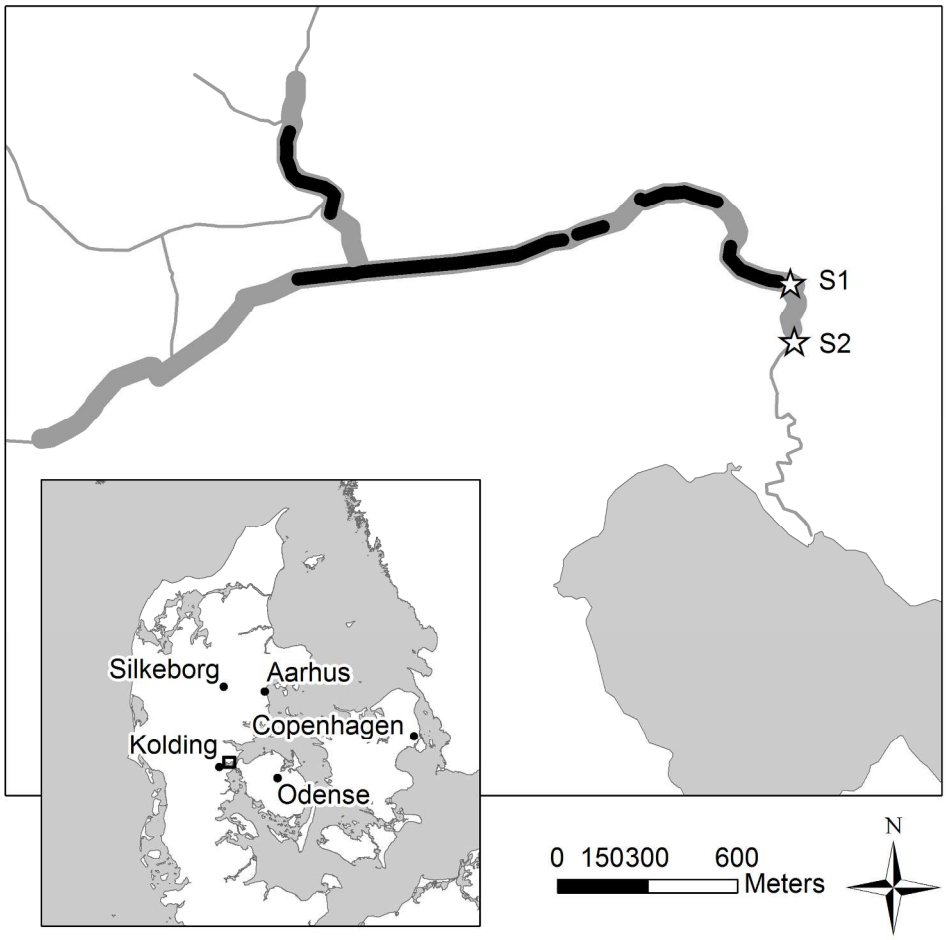
555 Table 2. Number of individuals recaptured, number of sampled individuals that were detected
556 at S1 and the mean cortisol concentration (with SD) of these individuals for baseline (sampled
557 within three minutes of capture) and post-stressor (sampled 30 min minutes following a
558 standardized three minute air exposure) treatment groups of juvenile brown trout (*Salmo*
559 *trutta*) collected from the Gudsø Stream, Denmark.

Treatment	Baseline	Post-Stressor
Number recaptured	13	6
Cortisol of recaptured (ng/mL)	5.6±5.0	69.9±40.1
Number S1 passage	37	46
Cortisol Passing S1(ng/mL)	5.8±7.7	76.2±42.3
Number S2 passage	19	26
Cortisol Passing S2 (ng/mL)	8.4±9.0	79.2±42.4

560

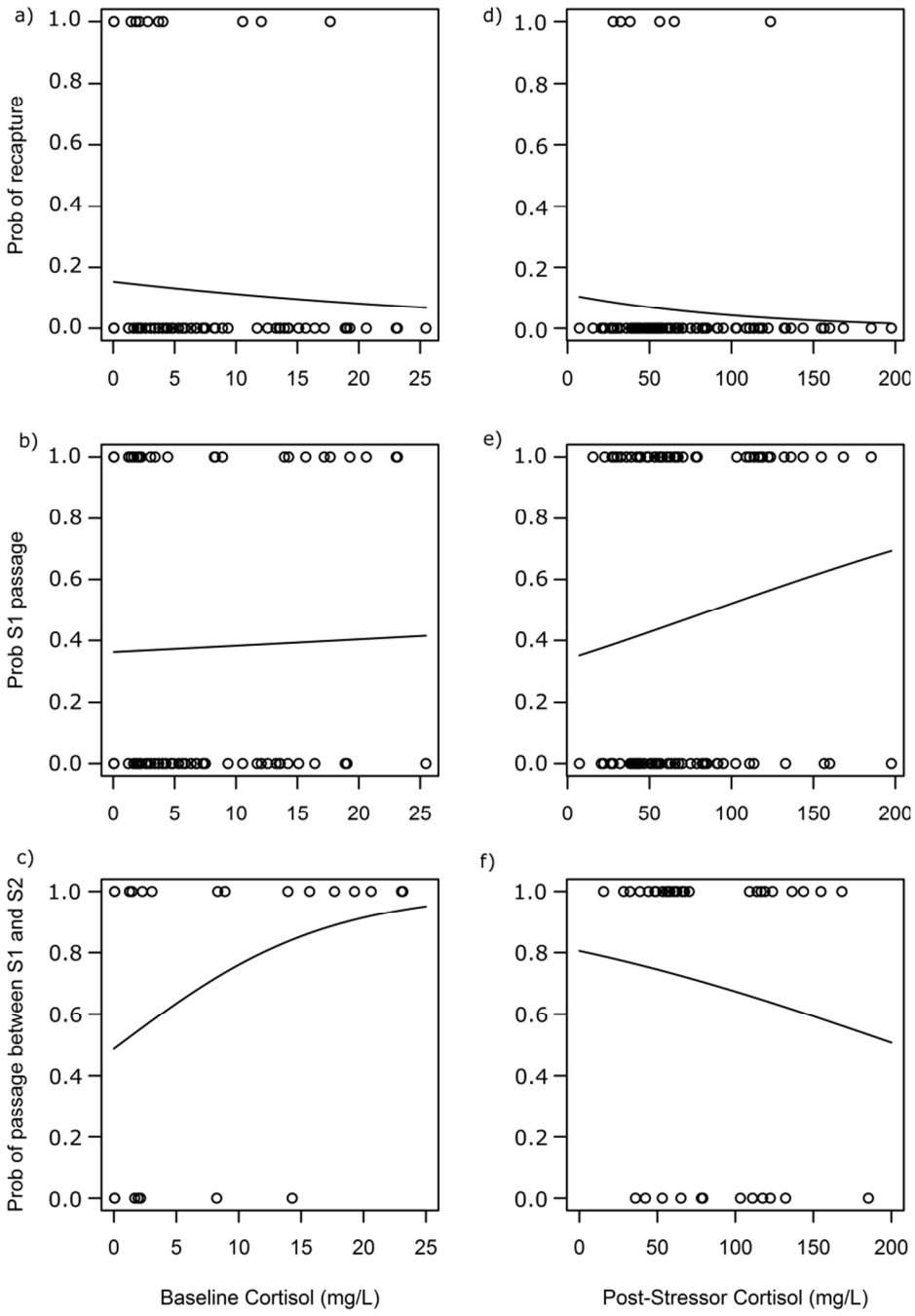
561

562 Figure 1. Location of Gudsø Stream in Denmark is shown as a black box in the bottom left panel
563 (north-east of Kolding). The portions of the Stream where brown trout (*Salmo trutta*)were
564 captured are shown as thick black lines and the areas re-sampled in June 2013 are shown as
565 thick grey lines. The positions of the two PIT antennas, S1 and S2, are also shown in the larger
566 map. Light gray areas represent water and the outflow location of Gudsø Stream into Kolding
567 Fjord. Map data from the European Environment Agency.
568
569 Figure 2. The probability of recapture (a), successful S1 passage (b), and successful passage
570 between S1 and S2 (c) as a function of individual baseline cortisol plasma concentration
571 collected within 3 minutes of capture, for individual juvenile brown trout (*Salmo trutta*) on the
572 Gudsø Stream, Denmark on the left, and on the right the probability of recapture (d), successful
573 S1 passage (e), and successful passage between S1 and S2 (f), as a function of individual cortisol
574 plasma concentration 30 minutes post 3 minute air exposure. Open circles represent actual
575 values found for individuals and the line represents the values predicted using a binary logistic
576 regression with no covariates. Binary logistic regression found no significant relationships
577 except for (c): increased baseline cortisol was associated with greater probability of successful
578 S2 passage for fish that had completed S1 passage, with marginal significance when mass
579 (p=0.05) or length (p=0.04) was included in the model as a covariate, but not when Fulton's
580 condition factor was included as a covariate. Significant relationships are indicated by three
581 stars.



582

583 Figure 1



584

585 Figure 2